Gene signature for prognosis in comparison of pancreatic cancer patient with diabetes and non-diabetes

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Background

Pancreatic cancer (PC) has very poor prognosis. And this cancer can be divided into diabetes and non-diabetes. Patients with these two types of PC differ at the molecular level.However,the gene biomarker for predicting prognosis of two types of PC is quite unclear yet.

Methods Both types of PC patients perform differently at the clinical and molecular levels. The Cancer Genome Atlas (TCGA) is employed in this study. The gene exprssion of the PC with diabetes and non-diabetes is used for predicting their prognosis by LASSO(Least Absolute Shrinkage and Selection Operator) Cox re-gression. Furthermore, the results are validated by exchange gene biomarker with each other and verified by independent Gene Expression Omnibus (GEO). The prognostic index (PI) is generated from gene biomarker combination that is used to rank the risk ratio of patients. Survival analysis is appiled to test significant difference between high-risk group and low-risk group.

Results An integrated gene prognostic biomarker consisted by 14 protective genes, among them 6 risky genes are identified in PC with non-diabetes. And another integrated gene prognostic biomarker consisted by 5 protective genes, there are 3 risky genes that are identified in PC with diabetes.Hence,the prognostic value of gene biomarker in PC with non-diabetes and diabetes are all greater than clinical traits (HR=1.102, p=2.68E-10; HR=1.212, p=2.83E-5).

Conclusions The results of this study indicated that the prognostic value of genetic biomarkers in PCs with non-diabetes and diabetes is greater than clinical traits.Therefore, this study are expected to provide a novel gene biomarker for predicting prognosis of PC with non-diabetes and diabetes and improving clinical decision.

Gene Signature for Prognosis in Comparison of Pancreatic Cancer patient with Diabetes and Non-diabetes

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Summplement Figure 1. The Cross-validation error curve of pancreatic cancer with diabetes.

Summplement Figure 2. The Cross-validation error curve of pancreatic cancer with non-diabetes.

Gene Biomarker for Prognosis in Comparison of PC patient with Diabetes and Non-diabetes

Abstract

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Conclusions

The results of this study indicated that the prognostic value of genetic biomarkers in PCs with non-diabetes and diabetes is greater than clinical traits.Therefore, this study are expected to provide a novel gene biomarker for predicting prognosis of PC with non-diabetes and diabetes and improving clinical decision.

Keywords: PC, diabetes, LASSO Cox regression, prognosis index

Introduction

PC is an aggressive cancer of the digestive system, which is becoming a serious health problem worldwide. Overall survival for patients with pancreatic cancer is poor, mainly due to a lack of biomarkers to enable early diagnosis and a lack of prognostic markers that can inform decision-making, facilitating personalized treatment and an optimal clinical outcome (1). Generally speaking, type-II diabetes frequently occurs in patients with PC .Thus, it is considered to be an important risk factor for malignancy of PC (2). In fact, PC with diabetes and without diabetes are very different in histopathology (3) and molecular level. Currently, many studies do not consider the difference between PC with diabetes and non-diabetes. They just considered that diabetes was a risk factor in PC development (4). With the deepening of people's understanding in the relationship between PC with diabetes and non-diabetes, recent data argues that diabetes and altered glucose metabolism are a consequence of PC, and yet, the clinical presentation of the altered glucose metabolism in these patients varies considerably (5). So, PC patients with diabetes and non-diabetes may represent two types of PC. Therefore, we predict that PC patients with diabetes and non-diabetes are also different in their prognostic biomarkers. The different prognostic biomarkers indicate that they should be treated via their own ways respectively.

In this study, The Cancer Genomic Atlas (TCGA) database and Gene Expression Omnibus (GEO) database were employed to investigate and validate gene biomarker for prognosis in PC with or without diabetes. By characterizing genetic alterations, TCGA project has provided a numberous amount of genomic cancer date and corresponding clincial data which we can be used to figure out the relationship between them of PC and that make us understand PC more better and more accurate. However, high through-put genomic data (microarray or High seq V2) may encounter the problem in statistics which called “curse of dimensionality’’(6). Due to this problem, ordinary regression is subject to over-fitting and instable coefficients, and stepwise variable selection methods do not

scale well (7). Therefore, the least absolute shrinkage and selection operator (LASSO) method is employed to resolve this problem(8,9). Through adjusting the coefficent of Cox regression, LASSO can penalize the regression in high dimensionality and colinearity to solve "curse of dimensionality"(10,11). Many studies have adopted elastic-net regression to screen genes,in order to predict cancer patinet survival. In the current study, we are going to subject the integrated mRNA and clinical factors profiles of PC patients，aiming to identify and analyze gene biomarker which can predict the overall survival (OS) in the diabetes and non-diabetes of PC patients by LASSO. Recently, many studies employed TCGA and GEO dataset to identify useful gene biomarker which can predicte prognosis in many various cancer patients (12,13). Along with the increasing genomic data of PC patients, lots of corresponding studies begin to analyze the genomic data and try their best to explorie a certan interesting and meaningful problems (14,15).

Materials and Methods

Information of Patients

All diabetic and non-diabetic patients with PC related studies were identified and collected by carefully searching the online TCGA databases (http://tcga-data.nci.nih.gov/tcga/). The following combination of keywords was simultaneously applied for the literature search according to the requirement of this study ‘pancreatic cancer ’or ‘PC’ or ‘pancreatic tumor’ or ‘pancreatic malignancy’ and ‘diabetes’ and ‘non-diabetes’ . In addition, the following research feature criteria are used to further improve and screen the desired search samples: (1) researches that concentrated on patients with diabetes and non-diabetes were selected; (2) survival time involved of patients was more than 30 days; (3) patients who didn't receive any adjuvant therapy before. (4) all tissues that are from patients must be the primary tumor. After filtering and screening the data by these above criteria, 136 samples were selected TCGA databases, which included 99 non-diabetic patients and 37 diabetic patients with PC.

RNA data Gathering and Filtering

The data of mRNA expression was downloaded from TCGA database. And the IIIuminaHiSeq RNASeqV2 platform is selected.

Clinical factors and survival analysis

Clinical factors for the both diabetic and non-diabetic patients with PC are listed detailedly in

supplementary table1.For the correlation between RNA expression and OS was carried out by forthputting univariate Cox regression (the two-sided log-rank test). In the present meta-analysis, HRs and corresponding 95% CIs were combined to estimate the value of cancer prognosis. The hazard ratio (HR) was calculated from exp (β) and β was the coefficient from Cox regression. Clinical variables from univariate Cox proportional hazards regression P value≤0.05 were regarded as a important indicator of diabetic and non-diabetic patient prognosis.

The Expression of mRNA associated with Survival Analysis

The relationship between patient survival and mRNA expression was analyzed through drawing on the univariate Cox proportional hazard regression. The null-selected RNA is calculated again and again. P value≤0.05 screened for mRNA($P \leq 0.05$).Generally speaking, RNAs that had a HR>1 and P value ≤0.05 were considered to be a risky gene while HR<1 is seen as a improved protective gene . In diabetic patients with PC, we reached a conclusion that 64 mRNAs are significantly associated with overall survival time ($p<0.05$) by univariate Cox regression. In non-diabetic patients with PC, we found that 1559 mRNAs are obvious significantly associated with overall survival time ($p<0.05$). In data of high dimension gene expression, the coefficients (β) of Cox regression model needs to be penalized in order that it can fit better and minimize errors as much as possible. Therefore, elastic net-regulated Cox regression method is applied to calculate the results from univariate Cox regression. The penalized log-likelihood function is defined as following:

$$l_p(\beta, X) = l(\beta, X) - \lambda \sum_{j=1}^{p} |\beta_j|$$

With the value of λ increasing, value of $\sum_{j=1}^{p} |\beta_j|$ would be decreased.Thence, some coefficients (β) of RNAs would be changed into 0. This result was analyzed by selecting the LASSO-adjusted Cox regression coefficient $\neq 0$ mRNA. These steps are carried out by R package “glmnet”. Finally,we obtained 8 mRNAs in diabetic patient with PC and 20 mRNAs in non-diabetic patients

with PC.

Prognosis index

PI is calculated from linear combination of candidate RNAs and their expression for each PC patient.We defined a weighted prognostic index (WPI) (16) for integrating indicators of RNAs for each PC patient, as following:

$$\mathrm{PI} = \sum(\beta i * Vi) \qquad (1)$$

$$\mathrm{WPI} = \frac{PI - mean\ (PI)}{SD(PI)} \quad (2)$$

Where β_i represents the coefficient in Cox regression of the *i*th variable. And V_i signifies the value of the *i*th variable. Mean (PI) and SD (PI) stand for the mean value and standard deviation of the PI, respectively. Where V_i is the expression value of each mRNA (log2-transformed expression value) and β_i is the LASSO regulated Cox proportional hazards regression coefficient of the *i*th RNA or clinical traits.

Risk stratification and ROC curves

The capacity of the integrated RNA and clinical model to predict clinical outcome was evaluated by comparing the analysis of area under curve (AUC) of the receiver operation characteristic (ROC) curves. AUC for the ROC curve was applied to the "*survivalROC*" package in R software(17). The higher AUC is considered as better model performance and range of AUC value is from 0.5 to 1. The AUC range from 0.80-0.90 is treated as good performance. And the range from 0.90-1.00 is considered to be excellent performance. The risk of patient group was classified into two groups by median of WPIs:high-risk and a low-risk. Survival analysis is forthputting Kaplan-Meier curves. Statistical analysis and graph in this study were using the software of R software(18), version 3.2.4 and Bioconductor, version 2.15 (19).

Gene Ontology and Pathway Enrichment

Gene ontology (GO) functional enrichment analysis was performed to RNAs which classified as low-risk and high-risk group by making use of the online tool of the DAVID (version 6.8). We chose "*Homo sapiens*" as the background in order to search terms "GO_TERM_BP_FAT" for further analysis. And the genes are also enriched in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for analysis(20).

Validation data of patient information collection

An independent mRNA expression data of PC patients with 65 PC patients was downloaded from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The clinical traits and expression were all downloaded from GSE62452. And the mRNA expression data were generated by Affymetrix Human Genome U133A Array.

Results

Clinical traits

In the TCGA PC cohort of the 136 patients, 99 patients are pancreatic patients without diabetes and 37 are PC with diabetes. And these data are summarized in table1. We calculated the clinical factors by adopting univariate survival analysis and multivariable Cox regression analysis. We chosed 9 clinical variables that includes age, gender, tumor status, alcohol history, history of chronic pancreatitis, number of lymph nodes positive, maximum tumor dimension, neoplasm histologic grade and pathologic stage. In pancreatic patients without diabetes cohort, tumor status is significantly associated with overall survival by long-rank and multivariate Cox regression analysis. This result indicated that tumor status is an independent factor correlated with overall survival. In pancreatic patients with diabetes cohort, gender is significantly associated with overall survival time. But this factor is not an independent factor by multivariate Cox regression analysis (Table 1).

Gene biomarker analysis in PC cohort

By analysis of non-diabetes and diabetes PC patients through LASSO Cox regression and multivariate Cox regression, we have gained 20 mRNAs and 8 mRNAs biomarker significantly association with overall survival respectively. Among these genes, the values of HR< 1 and P value <0.01 were considered as protective RNAs and otherwise the values of HR > 1 were risky RNAs (Table 2, 3). And the graph for elastic net Cox regression is listed in supplementary file (supplementary1 and supplementary2).

The PI was significantly associated with pancreatic patient survival. After normalized PI to WPI, the median value of WPI is acted as cutoff threshold to classify low-risk and high-risk patient cohort (Figure 1).

Validation of the prognostic biomarker

The results are employed two different ways to verify its stability and reliability. Firstly, we used the gene biomarker in PC patients with diabetes (8 mRNAs) to test the survival curve in PC patients with non-diabetes. Secondly, we used the gene biomarker in PC patients with non-diabetes (20 mRNAs) to swap above calculation.

The validated results showed that the gene biomarker in two groups performed poor result after exchange (Figure 2). The results indicated that the gene biomarker in different groups has specificity in each condition.

For validation result, independent mRNA expression data and corresponding clinical information of PC patient with non-diabetes is downloaded from GEO database to estimate the reproducibility and robustness of the results from TCGA database.

Gene Ontology Enrichment

The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 was employed to discover the function of genes both in PC patient with diabetes and non-diabetes. The eight genes in PC with diabetes were associated with regulation of transcription with a Benjamin correction p-value<0.05. And many genes had DNA binding function. For 20 genes identified in PC without

diabetes were not enriched statistically significant association.

Comparison of clinical traits and gene biomarker for predicting prognosis

We integrated clinical traits that significantly associated with survival and PI of gene biomarker that significantly associated with survival to analyze the pancreatic cancer in diabetic and non-diabetic individuals. After multivariate Cox regression analysis, the results showed that PI of gene biomarker performed greatest p-value (Table 4). We filtered the clinical factors that significantly associated with survival by log-rank test into integrative model. In PC with non-diabetes, tumor status, number of lymph nodes positive, stage G2, G3 and G4 were significantly associated with survival (Table 2). And in PC with diabetes, gender, stage G2 and G3 were significantly associated with survival by log-rank test (Table 2).

From the table, we find PI of gene biomarker have smallest p-value after multivariable Cox regression. Although HR is not the highest among clinical traits, p-value is the smallest. Besides, we can find that tumor status is another significant risk factor in PC with non-diabetes.

Discussion

In this study, we proposed two classes of gene biomarker in PC patients with and without diabetes which can guide us to predict PC patient survival more accurately. To a large extent,PC patients with and without diabetes have quite different gene biomarker for predicting prognosis. After a series of studies,we not only find that genes candidate in both PC patient groups have no overlapping but also figure out that gene biomarker in non-diabetes PC patients is validated by GEO database. Gene biomarker in diabetes PC patients data is not retrieved from GEO and literature. Thus, we just validated gene biomarker in non-diabetes PC patients.

The result indicates that the gene biomarker in both groups have been very specified. Therefore,they have their own gene biomarker for predicting their prognosis. Although a large number of studies have reported some biomarkers in PC patients, many genes are mainly identified in PC patients without diabetes. We identified and contrasted the markers for predicting two types

of PC patients. And many genes have not been reported yet by now. Of high risk prognostic genes, *CRCT1*, *MUC20*, *RTP1*, *C10orf111*, *SPACA5* and *FZD10* have high level of HR. *MUC20*, *FZD10* have been identified in PC patients (21,22) and these two genes play a vital role in two important pathways associated with cancer. *MUC20* participates in MET (Mesenchymal-Epithelial transitions) process which is a common process in many tumors (23). And it may regulate MET signaling cascade. It seems to decrease hepatocyte growth factor (HGF)-induced transient MAPK activation (24). *FZD10* is associated with WNT signaling pathway which is implicated in embryogenesis as well as in carcinogenesis (25). Other genes are not reported in PC patients. Only *SPACA5* is reported in bladder cancer (26). Although many genes have not been reported before, we find that these combinations of these genes can greatly distinct high-risk and low-risk PC patients with non-diabetes. Besides, these genes are validated in independent GEO database. The results of GSE62452 in GEO database show that these genes performed stability and the gene biomarker could distinct high-risk and low-risk gene greatly.

The gene biomarker in PC patients with diabetes, three genes are high-risk genes. We can find that the production of these three genes (*ZNF793*, *GBP6, FOSL1*) are binding function proteins. Thus, we infer that they are all transcription factors. Of the three genes, *FOSL1* has been reported to be closely associated with PC(27-29). But these studies have not reported that this high-risk gene is associated with PC with diabetes. Only one study reported that *FOSL1* is closely associated with diabetes mellitus(30). And this gene has not been identified in PC with non-diabetes. *GBP6* is reported in diabetes(31) but is not reported in PC patients with diabetes. *ZNF793* is not identified in both PC and diabetes. Thus, we infer that the gene is a potential risk factor in PC patients with diabetes.

By multivariate Cox regression analysis, interestingly, we find tumor status is an independent factor for predicting prognosis of PC patients with non-diabetes. And gender is an independent factor for predicting prognosis of PC patients with diabetes. Tumor status is a vital clinical factor for predincting prognosis in many cancers. However, gender as an independent indicator of PC patient

with diabetes is very difficult to understand. We expect more studies to find the reason.

From the results, we find that there is no overlapping of both groups. Thus, we infer that two types of PC would be very different in molecular level. Thus, two types of PC patients would be received different treatments. The gene biomarker in two types of PC is expected to provide a new drug target or new insight for improving clinical decision

Conclusion

In this study, we find that pancrtic cancer patients with diabetes and without diabetes have different gene markers for predicting their respective prognosis. Thus, the different gene marker might be as an useful tool for the clincial decision in future.

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Ethical Policies and Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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346 Table 1 Clinical traits in PC patients with non-diabetes and diabetes

Non-diabetes PC(n=99)				Diabetes PC(n=37)		
Factors	Death/patients	Log-rank	Multivariate Cox P	Death/patients	Log-rank	Multivariate Cox P
Age		0.051	0.496		0.959	0.446
<=64	22/52			7/16		
>64	31/47			8/21		
Gender		0.402	0.172		0.001*	0.340
Female	27/50			7/12		
Male	26/49			8/25		
Tumor Status		9.3e-06*	0.0004*		0.005*	0.513
With Tumor	42/57			10/17		
Tumor Free	6/35			2/15		
Unknown	7/7			3/5		
Alcohol history		0.537	0.144		0.599	0.638
Yes	40/68			10/27		
No	12/39			5/10		
Unknown	1/2			-		
History of chronic pancreatitis		0.597	0.998		0.273	0.998
Yes	4/8			3/4		
No	48/86			10/31		
Unknown	1/5			2/2		
Number of lymph nodes positive by he		0.003*	0.396		0.480	0.533
<3	22/52			7/20		
>=3	30/45			8/16		
Maximum tumor dimension		0.394	0.216		0.147	0.279
>3.5	27/44			9/16		
<=3.5	26/51			6/20		
Neoplasm histologic grade		0.039*			0.004*	
G1	4/16		-	2/7		-
G2	31/52		0.606	6/20		0.998

G3	17/29	0.202	7/10	0.308	
G4	1/2	0.757	-	-	
Pathologic stage		0.100		0.431	
Stage I	0/1	-	0/1	-	
Stage IA	1/3	0.997	0/1	0.998	
Stage IB	3/10	0.998	0/2	0.998	
Stage IIA	5/13	0.998	3/7	0.998	
Stage IIB	43/70	0.998	11/24	0.998	
Stage III	1/2	-	0/1	-	
Stage IV	-	-	1/1	-	

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Table 2 Gene biomarker in PC patients with non-diabetes

	Hazard	CI	P value	Description
Low Risk genes				
TTTY9B	0	0.000-0.028	0.0102	testis-specific transcript, Y-linked 9B (non-protein coding)
RNF121	0.001	0.000-0.260	0.0142	RING finger protein 121
FHAD1	0.006	0.001-0.051	3.60E-06	Forkhead-associated domain-containing protein 1
GTF2F2	0.007	0.000-0.516	0.0235	General transcription factor IIF subunit 2
ADAMTS19	0.009	0.001-0.113	0.0002	A disintegrin and metalloproteinase with thrombospondin motifs 19
LHFPL1	0.024	0.002-0.283	0.0031	Lipoma HMGIC fusion partner-like 1 protein
DHDH	0.05	0.013-0.191	1.16E-05	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase
LOC256880	0.062	0.006-0.600	0.0164	
SLC25A41	0.093	0.022-0.392	0.001	Solute carrier family 25 member 41
ZNF233	0.095	0.017-0.516	0.0060	Zinc finger protein 233
C6orf195	0.129	0.024-0.695	0.0171	
PCDHA11	0.144	0.050-0.419	0.00037	Proto cadherin alpha-11
LOC401127	0.146	0.022-0.969	0.0463	
TUBBP5	0.303	0.139-0.663	0.0028	tubulin beta pseudo gene 5
High risk genes				
CRCT1	2.107	1.154-3.847	0.0152	Cysteine-rich C-terminal protein 1
MUC20	14.76	4.387-49.66	1.37E-05	Mucin-20
RTP1	18.01	1.075-301.8	0.0444	Receptor-transporting protein 1
C10orf111	23.6	1.314-423.9	0.0319	
SPACA5	23.83	1.821-311.7	0.0156	Sperm acrosome-associated protein 5
FZD10	26.54	5.142-136.9	9.02E-05	Frizzled-10

354 Table 3 Gene biomarker in PC patients with diabetes

	Hazard	CI（95%）	p-value	Description
Low Risk genes				
SYS1-DBNDD2	0.347	0.909-1.815	0.0020	
NCRNA00167	0.231	0.978-1.719	0.0015	
IRX5	0.473	0.282-1.185	0.0012	Iroquois-class homeodomain protein IRX-5
ZNF77	0.244	0.770-1.801	0.0040	Zinc finger protein 77
CATSPERG	0.296	0.651-0.991	0.0029	Cation channel sperm-associated protein subunit gamma
High Risk genes				
ZNF793	2.968	0.358-1.978	0.0063	Zinc finger protein 793
GBP6	1.744	0.342-1.207	0.0011	Guanylate-binding protein 6
FOSL1	2.306	0.9601-1.051	0.0091	Fos-related antigen 1

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356

357 Table 4. Multivariate Cox regression analysis of prognosis index and clinical traits

PC with Non-diabetes	HR	CI	Multivariate Cox P-value
PI	1.102	1.070-1.136	2.68e-10*
Tumor Status	0.117	0.298-1.924	0.0005*
Number of lymph nodes positive by he	1.589	0.907-2.783	0.106
G2	2.103	0.187-5.400	0.123
G3	2.036	0.739-5.613	0.169
G4	2.215	0.257-19.087	0.469
PC with Diabetes			
PI	1.212	1.108-1.327	2.83e-05*
Gender	0.173	0.053-0.564	0.004*
G2	0.897	0.168-4.775	0.898
G3	5.310	0.892-31.616	0.067

358
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360 **Number of figures: 3**

361 **Figure 1. WPI analysis of the integrated gene-and-clinical model for 136 TCGA PC patients**.
362 (A) Survival analysis in PC patient with non-diabetes. (B) WPI distribution in the TCGA PC cohort
363 without diabetes. The dash line represents the cutoff used to categorize patients into the low-risk
364 group or the high-risk group. (C) Survival analysis in PC patient with diabetes. (D) WPI
365 distribution in the TCGA PC cohort with diabetes.
366
367 **Figure 2. Exchange gene biomarker to cross-validate in two groups**.(A) Using gene biomarker
368 of PC with diabetes to test in PC with non-diabetes. (B) Using gene biomarker of PC with non-
369 diabetes to test in PC with diabetes
370
371 **Figure 3. Kaplan-Meier curves and ROC curves for validation PC patients in GEO database.**
372 (A)The gene biomarker can greatly classifiy PC patients into high-risk and low-risk groups
373 ($p<0.001$). (B)The AUC of ROC is 0.828, which represent that the gene biomarker model is very
374 good.
375

Supplementary File legend

Figure S1. The Cross-validation error curve of PC with diabetes. The left vertical dotted line reveals the partial likelihood deviance achieves its minimum lambda, which represents a fairly regularized model. The right vertical dotted line indicates the most regularized model (ie, null model) with cross-validation error within one standard deviation of the minimum. The numbers at the top of the figure indicate the number of nonzero coefficients.

Figure S2. The Cross-validation error curve of PC with non-diabetes. The left vertical dotted line reveals the partial likelihood deviance achieves its minimum lambda, which represents a fairly regularized model. The right vertical dotted line indicates the most regularized model (ie, null model) with cross-validation error within one standard deviation of the minimum. The numbers at the top of the figure indicate the number of nonzero coefficients

Table 1(on next page)

Clinical traits in PC patients with non-diabetes and diabetes

Table 1 Clinical traits in PC patients with non-diabetes and diabetes

Non-diabetes Pancreatic Cancer(n=99)				Diabetes Pancreatic Cancer(n=37)		
Factors	Death/patients	Log-rank	Multivariate Cox P	Death/patients	Log-rank	Multivariate Cox P
Age		0.051	0.496		0.959	0.446
<=64	22/52			7/16		
>64	31/47			8/21		
Gender		0.402	0.172		0.001*	0.340
Female	27/50			7/12		
Male	26/49			8/25		
Tumor Status		9.3e-06*	0.0004*		0.005*	0.513
With Tumor	42/57			10/17		
Tumor Free	6/35			2/15		
Unknown	7/7			3/5		
Alcohol history		0.537	0.144		0.599	0.638
Yes	40/68			10/27		
No	12/39			5/10		
Unknown	1/2			-		
History of chronic pancreatitis		0.597	0.998		0.273	0.998
Yes	4/8			3/4		
No	48/86			10/31		
Unknown	1/5			2/2		
Number of lymph nodes positive by he		0.003*	0.396		0.480	0.533
<3	22/52			7/20		
>=3	30/45			8/16		
Maximum tumor dimension		0.394	0.216		0.147	0.279
>3.5	27/44			9/16		
<=3.5	26/51			6/20		
Neoplasm histologic grade		0.039*			0.004*	
G1	4/16		-	2/7		-
G2	31/52		0.606	6/20		0.998
G3	17/29		0.202	7/10		0.308
G4	1/2		0.757	-		-
Pathologic stage		0.100			0.431	
Stage I	0/1		-	0/1		-
Stage IA	1/3		0.997	0/1		0.998
Stage IB	3/10		0.998	0/2		0.998

Stage IIA	5/13	0.998	3/7	0.998
Stage IIB	43/70	0.998	11/24	0.998
Stage III	1/2	-	0/1	-
Stage IV	-	-	1/1	-

Table 2(on next page)

Gene signature in PC patients with non-diabetes

Table 2 Gene signature in PC patients with non-diabetes

	Hazard	CI	P value	Description
Low Risk genes				
TTTY9B	0	0.000-0.028	0.0102	testis-specific transcript, Y-linked 9B (non-protein coding)
RNF121	0.001	0.000-0.260	0.0142	RING finger protein 121
FHAD1	0.006	0.001-0.051	3.60E-06	Forkhead-associated domain-containing protein 1
GTF2F2	0.007	0.000-0.516	0.0235	General transcription factor IIF subunit 2
ADAMTS19	0.009	0.001-0.113	0.0002	A disintegrin and metalloproteinase with thrombospondin motifs 19
LHFPL1	0.024	0.002-0.283	0.0031	Lipoma HMGIC fusion partner-like 1 protein
DHDH	0.05	0.013-0.191	1.16E-05	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase
LOC256880	0.062	0.006-0.600	0.0164	
SLC25A41	0.093	0.022-0.392	0.001	Solute carrier family 25 member 41
ZNF233	0.095	0.017-0.516	0.0060	Zinc finger protein 233
C6orf195	0.129	0.024-0.695	0.0171	
PCDHA11	0.144	0.050-0.419	0.00037	Proto cadherin alpha-11
LOC401127	0.146	0.022-0.969	0.0463	
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Table 3(on next page)

Multivariate Cox regression analysis of prognosis index and clinical traits

Table 3 Gene signature in PC patients with diabetes

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